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Splicing Regulation: The Cell Cycle Connection

Dispatch

Benjamin J. Blencowe

Many genes are repressed during mitosis, and this is known to involve differential phosphorylation of specific factors required for transcription, 3′-end RNA processing and translation. A recent study suggests that splicing is also targeted for mitotic repression, in this case by dephosphorylation of the newly identified splicing factor SRp38.

During the past two decades great strides have been made in elucidating many of the molecular controls that underlie progression through the cell cycle. Perhaps not surprising for a process that requires such drastic reprogramming of cell architecture, a major transition that occurs during mitosis is the arrest of much of the gene expression that is active during interphase. Transcription, polyadenylation and translation are all known to be targets for this mitotic inhibition, in each case by a change in the phosphorylation status of one or more factors required for these processes [1–5]. Shin and Manley [6] have now reported evidence that pre-mRNA splicing is also a target for mitotic inhibition. The mitosis-regulated dephosphorylation of SRp38, a recently identified member of the SR family of splicing factors, appears to be involved in this inhibition. This study contributes interesting and important new information relevant to cell-cycle control, and also raises intriguing questions as to the mechanisms and specificity of regulation of splicing during the cell cycle.

Numerous earlier studies have established connections between pre-mRNA splicing and the cell cycle (reviewed in [7]). For example, conditional mutations in several splicing factors in yeast cause cell-cycle arrest at the restrictive temperature, although in most cases it was not clear whether the effects were direct or indirect. In one recent study [8], however, it was shown that cell-cycle arrest at the G2/M transition in the budding yeast *Saccharomyces cerevisiae*, as a result of a temperature-sensitive mutation in the conserved spliceosome-associated protein Cef1p, could be partially suppressed by the deletion of the intron in the α tubulin gene. So in this case, and probably many others involving genetic defects in splicing components that impact on the cell cycle, the arrest most likely is indirect, caused by the disruption of the splicing of one or more pre-mRNAs required for cell-cycle progression.

Other studies have indicated a link between the mammalian kinase SRPK1 and its homologs in other species — Dsk1 in the fission yeast *Schizosaccharomyces pombe* and Sky1 in *S. cerevisiae* — and the cell cycle. Dsk1 was first identified as a kinase that, when overproduced, prevents cell-cycle progression [9]. SRPK1 was isolated as a kinase that is upregulated during mitosis and induces the disassembly of splicing-factor-rich nuclear 'speckles' (also referred to as interchromatin granule clusters) [10] (Figure 1). SRPK1 and its homologs were subsequently shown to phosphorylate serine and threonine residues within the RS (rich in alternating arginine and serine residues) domains of splicing factors, including members of the SR family [11]. RS domains mediate protein–protein interactions with other RS domains, and these interactions are important for the assembly of multisubunit complexes required for both constitutive and regulated splicing (reviewed in [12]). From previous work on SRPK1, and from the more recent work on SRp38 described below [6], differential phosphorylation of RS domains appears to be important for one or more aspects of splicing regulation during the cell cycle.

SR family splicing factors have a similar domain organization, with one or two amino-terminal RNA recognition motifs (RRMs) and a carboxy-terminal RS domain (reviewed in [12]). Members of this family identified earlier also have several common functional properties: for example, each can activate splicing when added to *in vitro* reactions in a cytoplasmic S100 extract (which contains all the components necessary for splicing activity except for SR family proteins and other RS domain splicing factors). Although SRp38 has a similar domain organization (Figure 2A), it does not have the activator function of other family members: when recombinant SRp38 was added to splicing reactions, surprisingly it was found to inhibit pre-mRNA splicing [6,13].

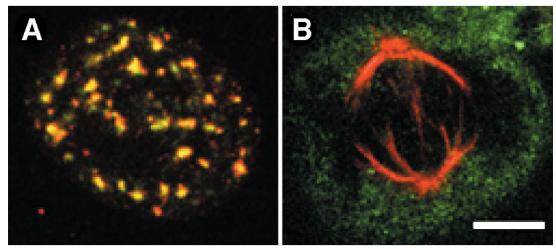

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Figure 1. Redistribution of spliceosomal components during mitosis.

(A) Confocal microscope image of an interphase human CaSki cell double-immunolabeled with antisera specific for SRm160 (red) and SRm300 (green), two RS domain proteins associated with spliceosomes. Both proteins concentrate in splicing factor-rich nuclear speckles during interphase; regions of overlap between the two proteins are indicated (yellow). (B) During mitosis nuclear speckle structures disassemble and many splicing factors, such as SRm300, disperse and/or concentrate in mitotic interchromatin granules. The confocal image shows a single CaSki cell at metaphase, double immunolabeled with anti-SRm300 (green) and anti-tubulin (red) antibodies. Bar = 5μm. (Images reproduced from [14].)

Benjamin J. Blencowe, C.H. Best Institute, University of Toronto, 112 College Street, Room 410, Toronto, Ontario, Canada M5G 1L6. Email: b.blencowe@utoronto.ca

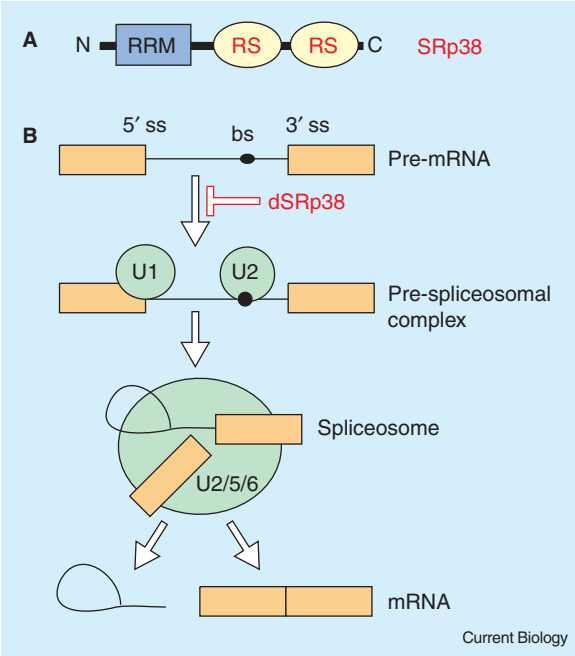

Figure 2. Mitotic inhibition of splicing by dSRp38.
(A) Domain structure of SRp38. Like other SR proteins, SRp38 contains an amino-terminal RNA recognition motif (RRM) and a phosphorylated carboxy-terminal alternating arginine/serine-rich (RS) domain. (B) An unidentified phosphatase that is activated during mitosis dephosphorylates SRp38, generating the dephosphorylated form dSRp38 which inhibits splicing at an early step, prior to the formation of a pre-spliceosomal complex containing U2 snRNP [6]. 5′ ss, 5′ splice site; 3′ ss, 3′ splice site; bs, branch site.

Shin and Manley [6] noted that SRp38 migrates in cell extracts as a doublet. As the RS domains of SR proteins are known to be phosphorylated by kinases such as SRPK1, it was suspected that the lower form of the protein corresponded to dephosphorylated SRp38 (dSRp38). This was confirmed by phosphatase treatment, which converted all of the SRp38 in the extract to the lower-migrating form. Strikingly, it was found that preparations of dSRp38 protein were approximately 50-fold more active at inhibiting splicing than preparations consisting predominantly of the phosphorylated form of the protein.

SRp38 thus appears to be unusual among SR family proteins in that it acts as a splicing repressor, triggered by dephosphorylation by an as yet unidentified phosphatase. Although the mechanism by which dSRp38 causes splicing inhibition is not clear, it was observed that dSRp38 does not interact stably with other SR proteins, arguing against a model in which it prevents splicing by direct disruption of interactions involving SR proteins that function in splicing. Addition of the protein to splicing reactions was, however, observed to prevent the formation of early splicing complexes containing the spliceosome component U2 snRNP (which binds to the intron branch-point region of the pre-mRNA splicing substrate), a step in spliceosome assembly which normally requires SR proteins [6] (see Figure 2B).

Shin and Manley [6] conjectured that one step at which dephosphorylation of SRp38 might play an important regulatory role is mitosis, given the precedent that other steps in gene expression are inactivated by a change in phosphorylation status of one or more critical components. To investigate this possibility, they initially compared the phosphorylation status of SRp38 at different stages during the cell cycle, using extracts from cells synchronized after release from a double-thymidine block: higher levels of dSRp38 were detected as cells entered mitosis.

Shin and Manley [6] next performed an elegant series of experiments to test whether splicing, like other steps in gene expression, is blocked at mitosis, and also whether dSRp38 might be involved in such a block. They prepared whole-cell extracts using three different salt extraction conditions — 0.3M, 0.6M and 2.0M — from cells grown asynchronously (AE) or after mitotic arrest (ME). When these three extracts were tested for splicing activity, both the AE and ME prepared using 0.3M salt were active; but splicing was specifically and increasingly inhibited in the ME when the extractions were performed at the higher salt concentrations. Remarkably, this inhibition correlated perfectly with the increased presence of dSRp38 in the ME, which was only released from the insoluble fraction of the extracts at the elevated salt concentrations.

To test whether dSRp38 might be responsible for the ME block, Shin and Manley [6] depleted most of the protein by using avidin beads coupled to an RNA fragment selected to bind with high affinity to the RRM of SRp38. Strikingly, they found that splicing was activated in the 2M salt-extracted ME depleted of SRp38. Moreover, splicing inhibition was restored to this depleted extract by addition of dSRp38, but not by SRp38 or the SR family protein ASF/SF2.

Aside from the issue of whether these results reflect a physiologically important mechanism for mitotic inhibition of splicing, which was not addressed by Shin and Manley [6], there are some aspects of the results that bear consideration for future studies in this area. In particular, while dSRp38 may appear to be unique in its ability to prevent splicing *in vitro*, the results do not in any way exclude the possibility that other splicing factors, especially those with RS domains, are also targets for splicing regulation during the cell cycle.

First, there is the issue of functional redundancy among SR proteins to consider (reviewed in [12]). Previous studies found that depletion of individual RS domain proteins can prevent splicing of different pre-mRNA substrates, but addition to the depleted extracts of SR proteins other than the specifically depleted component can often restore splicing activity. This is most likely because SR proteins, at endogenous levels, function by forming multiple weak, cooperative interactions which are required to assemble splicing complexes. Specific depletion of any one factor contributing to such interactions may be sufficient to prevent splicing; but addition of excess levels of any of a number of SR proteins may be sufficient to restore activity, because of the redundant nature of the interactions that can form at elevated levels of these proteins.

The situation with dSRp38 may be similar: at endogenous concentrations, it may repress splicing in the context of weak, cooperative interactions that are lost when it is depleted. But other RS domain proteins, which may also have an altered phosphorylation status at mitotis, might also be able to repress splicing. Although Shin and Manley [6] did not detect substantial changes in the phosphorylation status of other SR proteins in mitotic-phase extracts, the monoclonal antibody they used (mAb104) may simply fail to detect many other RS domain proteins, several of which are candidates for having cell-cycle-associated functions [14,15]. Moreover, SRp38 is not ubiquitously expressed [6], suggesting that there are other factors which suppress splicing during mitosis in the tissues that lack SRp38.

A related issue is the unresolved role of SRPK1 and its homologs during the cell cycle. Earlier work suggested that SRPK1 and its *S. pombe* homolog Dsk1 might participate in mitosis [9,10]. While the phosphatase which targets SRp38 during mitosis is not known, it is interesting to consider that it might act on serine and threonine resides in the RS domain of the protein and that SRPK1 and/or related kinases might function to regenerate phosphorylation of dSRp38, and possibly other RS domain proteins, and thereby allow a return to interphase splicing patterns upon exit from mitosis.

There also remains the more general question of why splicing is inhibited during the cell cycle, particularly given that three other steps in gene regulation are targeted for mitotic inhibition. One possibility is that blocking gene expression at multiple levels ensures limited competition for cell-cycle-specific functions. Although splicing inhibition during mitosis might be largely redundant with these other blocks, it may nevertheless be important to ensure that processed transcripts that are not required, or which might inhibit cell-cycle progression, are not made. Sequestration of unprocessed transcripts in mitotic complexes by factors such as dSRp38 might facilitate such a block. These transcripts might then be available for rapid expression following release of the block during exit from mitosis.

Related to these issues is the question of the extent of splicing regulation during different stages of the cell cycle. For example, it will be important to determine which changes in alternative splicing regulation, in addition to transcriptional control, are required for cell-cycle progression. Clearly, much work needs to be done to elucidate the nature of the molecular mechanisms which function to regulate splicing as well as other steps in gene expression during the cell cycle.

References

1. Colgan, D.F., Murthy, K.G., Prives, C. and Manley, J.L. (1996). Cell-cycle related regulation of poly(A) polymerase by phosphorylation. Nature *384*, 282-285.
2. Segil, N., Guermah, M., Hoffmann, A., Roeder, R.G. and Heintz, N. (1996). Mitotic regulation of TFIID: inhibition of activator-dependent transcription and changes in subcellular localization. Genes Dev. *10*, 2389-2400.
3. Akoulitchev, S. and Reinberg, D. (1998). The molecular mechanism of mitotic inhibition of TFIIH is mediated by phosphorylation of CDK7. Genes Dev. *12*, 3541-3550.
4. Long, J.J., Leresche, A., Kriwacki, R.W. and Gottesfeld, J.M. (1998). Repression of TFIIH transcriptional activity and TFIIH-associated cdk7 kinase activity at mitosis. Mol. Cell Biol. *18*, 1467-1476.
5. Pyronnet, S., Dostie, J. and Sonenberg, N. (2001). Suppression of cap-dependent translation in mitosis. Genes Dev. *15*, 2083-2093.
6. Shin, C. and Manley, J.L. (2002). The SR protein SRp38 represses splicing in M phase cells. Cell *111*, 407-417.
7. Burns, C.G. and Gould, K.L. (1999). Connections between pre-mRNA processing and regulation of the eukaryotic cell cycle. Front. Horm. Res. *25*, 59-82.
8. Burns, C.G., Ohi, R., Mehta, S., O'Toole, E.T., Winey, M., Clark, T.A., Sugnet, C.W., Ares, M., Jr. and Gould, K.L. (2002). Removal of a single alpha-tubulin gene intron suppresses cell cycle arrest phenotypes of splicing factor mutations in Saccharomyces cerevisiae. Mol. Cell Biol. *22*, 801-815.
9. Takeuchi, M. and Yanagida, M. (1993). A mitotic role for a novel fission yeast protein kinase dsk1 with cell cycle stage dependent phosphorylation and localization. Mol. Biol Cell. *4*, 247-260.
10. Gui, J.-F., Lane, W.S. and Fu, X.-D. (1994). A serine kinase regulates intracellular localization of splicing factors in the cell cycle. Nature *369*, 678-682.
11. Gui, J.-F., Tronchere, H., Chandler, S.D. and Fu, X.-D. (1994). Purification and characterization of a kinase specific for the serine- and arginine-rich pre-mRNA splicing factors. Proc. Natl. Acad. Sci. U.S.A. *91*, 10824-10828.
12. Graveley, B.R. (2000). Sorting out the complexity of SR protein functions. RNA *6*, 1197-1211.
13. Cowper, A.E., Caceres, J.F., Mayeda, A. and Screaton, G.R. (2001). Serine-arginine (SR) protein-like factors that antagonize authentic SR proteins and regulate alternative splicing. J. Biol Chem. *276*, 48908-48914.
14. Blencowe, B.J., Bauren, G., Eldridge, A.G., Issner, R., Nickerson, J.A., Rosonina, E. and Sharp, P.A. (2000). The SRm160/300 splicing coactivator subunits. RNA *6*, 111-120.
15. Boucher, L., Ouzounis, C.A., Enright, A.J. and Blencowe, B.J. (2001). A genome-wide survey of RS domain proteins. RNA *7*, 1693-1701.